DATA EVALUATION RECORD
HONEY BEE - FIELD TESTING FOR POLLINATORS, §141-5

1. **CHEMICAL:** Novaluron — PC Code No.: 124002

2. **TEST MATERIAL:** "RIMON" 10EC — Purity: 9.7%

3. **CITATION:**

Author:	Barrett, K.
Title:	Novaluron: To Assess the Effect of "RIMON" 10EC to Honeybee Brood When Used in Commercial Citrus Groves
Study Completion Date:	March 19, 2001
Laboratory:	Huntingdon Life Sciences Ltd. Huntingdon , Cambridgeshire, England
Sponsor:	Makhteshim Chemical Works Ltd. Beer-Sheva, Israel
Laboratory Report ID:	MAK 542/993030
DP Barcode:	D285479
MRID No.:	45638409

4. **REVIEWED BY:** Rebecca Bryan, Staff Scientist, Dynamac Corporation.

Signature: Rebecca Bryan **Date:** 4/1/03

APPROVED BY: Teri Myers, Ph.D., Staff Scientist, Dynamac Corporation

Signature: Teri Myers **Date:** 4/1/03

5. **APPROVED BY:** Bill Evans, ERB I

Signature: Bill Evans **Date:** 11/24/03

6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Apis mellifera*
Definitive Study Duration: March 23-May 30, 1999 (68 days)

7. CONCLUSIONS:

This study examined the effect of "RIMON" 10EC on the honey bee colony brood development in commercial Shamouti Orange groves. Four replicate beehives (about 20,000 bees each) were centrally placed in duplicate 1 ha control and treatment plots that were sprayed (unshielded) with test solutions on two application periods at 7-day intervals during flowering. The negative control plots were sprayed with water and the treatment group plots were sprayed at a maximum nominal exposure concentration of 225 g a.i./ha "RIMON" 10EC. A reference toxicant, Dimilin WP, was tested at a nominal concentration of 1500 g a.i./ha. All plots were located at least 5-6 km apart to avoid the chance of cross-foraging.

On the first day of treatment and seven days after treatment, hives were assessed to determine percent eggs, young larvae, and old larvae. Prior to the second application and on days 18-20 of bee development, the % eggs were also assessed. In addition to these endpoints, bee brood development, health and hive condition were assessed. Numbers of dead bees (from dead bee traps) were counted and samples of pollen were collected to evaluate foraging activity and for residue analysis. At study termination, combs of honey were taken from each hive for residue analysis. After the final evaluation, hives were transferred to a separate location for a month for post-treatment monitoring.

The study author's analysis detected significant effects ($p<0.05$) of "RIMON" 10 EC on egg development, young larvae, and old larvae following the first application of "RIMON" 10EC, compared to the control. However, where eggs, young or old larvae had been removed, the second generation eggs appeared to be developing normally. There was no effect on the survival or foraging activity of adult bees in the colony. There were no effects on the long-term viability of the hive (one month post-treatment); numbers of bees increased in all hives and foraging activity appeared to be unaffected. Chemical analysis of pollen confirmed that bees had foraged in the treated area and that bees foraging in control plots did not forage in treated plots. No residues were found in honey from either control or "RIMON" 10EC-treated plots. The LC_{50} could not be determined, but it was presumed to be >225 g a.i./ha.

The results of this study imply that the adverse effects of "RIMON" 10EC on honeybee brood development are transient, affecting a portion of the brood eggs, young and old larvae immediately following spraying. There is not enough evidence to suggest that treatment with "RIMON" 10 EC adversely affects hive viability, pollination efficiency, or productivity of worker honeybees.

The study is classified as Supplemental because there is not an EPA-approved protocol for this type of study. The study is scientifically sound and the information that it provides may be useful for risk assessment purposes.

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: These studies are only required on a case-by-case basis. A protocol was not approved by the EPA for this insect field study, but it provides useful information for risk assessment purposes.

C. Repairability: None

9. GUIDELINE DEVIATIONS: N/A

10. SUBMISSION PURPOSE: This study was submitted to evaluate the effect of "RIMON" 10EC on the honey bee colony brood development in commercial citrus groves.

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: Species of concern (*Apis mellifera*, *Megachile rotundata*, or *Nomia melanderi*)	*Apis mellifera*
Age at beginning of test:	Colonies with all life-stages present. Hives were set up approximately 10 days prior to treatment.
Pre-test health:	Hives were healthy, well fed, and queen-right colonies.

Guideline Criteria	Reported Information
Supplier	Bee Keeping Division, State of Israel, Ministry of Agriculture and Rural Development, Extension Service, Tel Aviv, Israel.
Hive description:	Frames within each hive comprised 3-4 combs of brood at various stages of development, 2-3 brood-less combs with honey and 0.5-1 combs of pollen.
All bees from the same source?	Yes

B. Test System

Guideline Criteria	Reported Information
Site Characterization:	• The test plots were 1 ha in size and located in commercial Shamouti orange groves in Israel; site description details provided on p. 12. • There were two replicate, 1 ha plots for each experimental level (negative control, treatment, and reference groups). • The test plots were 5-6 km apart to reduce contamination from foraging. The distance between trees was 4-5 m and the distance between rows was 5-6 m. • No pesticide applications other than treatment for the test were applied to the plots. • Temperature (air), relative humidity, and precipitation events were recorded hourly using a portable weather station.

Guideline Criteria	Reported Information
Number of Plots/Treatment:	Two, 1 ha plots per treatment group, each with four replicate hives centrally-located within the pre-marked 1 ha trial plot. Brood development for each replicate was determined from the 100 cells with eggs, 100 cells with young larvae (2-3 days old), and 100 cells with old larvae (6-7 days old) designated on day one of treatment. An additional group of 100 eggs was designated on day of the second application.
Trap descriptions:	Dead bee traps were wooden boxes (46.5 x 18 x 12 cm) with a plastic filter net (0.5 cm^2 holes). These traps were placed in front of two replicate bee hives per plot so bees exiting the hive were required to pass through the wire mesh, which prevented live worker bees from carrying dead bees away from the hive. The pollen traps were made of a metal screen mesh, which removed pollen carried by returning worker bees. These traps were placed in front of the remaining two replicate hives on each plot.
Food Preparation:	The bees were allowed to forage for natural surrounding nectar sources prior to treatment and during testing. The pollen samples indicate that other plant sources besides the treated orange crop were also being foraged.
Precipitation:	0-5.2 mm (mean of 0.2 mm)
Temperature:	Air: 5.0-31°C (mean of 16.6°C)

Guideline Criteria	Reported Information
Wind speed:	0-4.782 m/s (mean of 0.745 m/s)
Relative humidity:	12.7-98.9% (mean of 74.3%)

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	No
Reference toxicant tested?	Yes, diflubenzuron 250 g/kg (Dimilin WP)
Application Rate	225 g a.i./ha (recommended field rate)

Guideline Criteria	Reported Information
Method of administration:	• The test substance,"RIMON" 10EC, was dispersed in water to obtain 225 g a.i./ha. The toxic reference product, Dimilin WP, was applied at a rate of 1500 g a.i./ha. A control treatment of water was applied at a volume of 3000 L/ha. • 3000 L/ha of each treatment was sprayed in the orange grove plots using commercial air blast equipment (sprayer, spray tank, and tractor); details regarding the sprayer and application are provided on p. 14. • Two applications were made of each treatment at 7 day intervals. The actual volumes of solution applied to each plot were within 15% of nominal over the 4 application days (April 4, 5, 11, and 12, 1999). Because of the time required to spray each plot and to travel between plots, treatments could not be completed in a single day and were split over two days, with replicate plots treated on subsequent days. • To minimize the risk of cross contamination, applications were made in the order of water control, "RIMON" 10EC, and Dimilin WP. The sprayer was rinsed with water between chemical treatment applications and washed extensively between successive application days.

Guideline Criteria	Reported Information
Sufficient number of time periods to yield statistically sound data?	Yes, Colonies were observed between April 4, 1999-May 30, 1999.
Controls: Negative control and/or diluent/solvent control	Negative (water) control
Number of colonies per group:	There were four replicate hive colonies (approximately 20,000 bees each) in each treatment and control plot. There were duplicate plots for each experimental group. In total, there were eight hive colonies per experimental group.
Solvent: Distilled water or the following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	N/A

Guideline Criteria	Reported Information
Observations and frequency:	• All honeybee hives were inspected one day prior to treatment to assess the pre-treatment hive strengths. This included assessing each frame in each hive for the proportion of cells containing different brood stages (i.e., eggs, larvae and pupae), honey (including open and closed cells), and pollen. Dead bee and pollen traps were also emptied. • Brood development was observed on day 7 after treatment. At an age of approximately 20 days, the pupa were removed and their development and age were assessed. On the day of the second application, an additional group of at least 100 eggs was assessed; development of these eggs was assessed 17 days later. • Mortality in dead-bee traps were observed periodically (every 2-4 days) and pollen was collected from traps at the same times. • Colony assessments were made at regular intervals, noting the proportion of cells containing different brood stages (i.e., eggs, larvae and pupae, honey, and pollen). • Assessment of foraging activity on the treated crop was not possible, but flowers were available for bees to forage in all plots and bees were audible in the treated

Guideline Criteria	Reported Information
Observations and frequency (cont.):	• Residues of "RIMON" 10EC in the hive and bees were determined from pupae that were extracted on day 17. In addition, frames of honey were removed at the end of the study. • Samples of pollen were collected during the study and analyzed to confirm if the bees were foraging in the treated area.

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control performance:	Bees from control hives showed either a similar or increased response compared to bees from the "RIMON" 10EC-treated hives. Evaluation of pollen collected from hives confirmed that bees were foraging extensively, up to 60%, on the pollen in the control plots, compared to up to 94% in the "RIMON" 10EC-treated plots and 40% in the Dimilin-treated plots. Furthermore, analysis of pollen samples detected Novaluron in samples collected from plots treated with "RIMON" 10EC and there was no evidence of Novaluron in the control samples of pollen, indicating that there was no cross-foraging by worker bees on the other treated plots in the study.

Guideline Criteria	Reported Information
Chemical analysis:	Pollen: Novaluron levels in pollen ranged between 0.01 and 0.1 mg/kg. Levels peaked following the second application of "RIMON" 10EC and declined in subsequent samples. Honey: Novaluron levels in honey were either below the limit of quantitation (0.01 mg/kg) or not detected in all samples taken from hives located in "RIMON" 10EC treated plots. Pupae: There were low levels of Novaluron (0.01 mg/kg) in two samples extracted at approximately day 17 of development from hives of "RIMON" 10EC treated plots. These were both from "old larvae". A third sample derived from the first set of eggs contained levels below the limit of quantitation. The remaining 22 samples contained no detectable residues of Novaluron.
Raw data included?	Yes
Signs of toxicity (if any) were described?	Developmental anomalies of exposed honeybee colonies were recorded with the date of observations. Post-study monitoring of hives revealed no adverse effects after one month, with the number of bees increased in all hives and foraging activity unaffected.

Mortality

Group (g a.i./ha "RIMON" 10EC) Nominal Concentration	Number of dead bees				
	Replicate * 1	Replicate 2	Replicate 3	Replicate 4	Total
Control (water)	57	31	19	12	119
225	49	52	28	4 + >20	133 + >20
Reference, Dimilin WP, 1500 g a.i./ha	32	29	10	11	82

* The control replicates correspond to hive numbers 1, 3, 2, and 4, respectively. The "RIMON" 10EC replicates correspond to hive numbers 2, 4, 2, and 4, respectively. The Dimilin WP replicates correspond to hive numbers 1, 3, 1, and 3, respectively. Data were obtained from Appendix 8 and totals exclude dead bees from traps during the pre-spray period.

Successful Development (Mean % of 100 bees selected at test initiation)

Group (g a.i./ha "RIMON" 10EC) Nominal Concentration	Time point*	Replicate	Life Stage			
			Eggs	Young larvae	Old larvae	Second spray eggs
Control (water)	1	Plot 1	86.46	95.81	95.96	89.35
		Plot 2	93.41	92.13	98.10	95.22
	2	Plot 1	70.96	91.94	91.92	83.81
		Plot 2	87.05	87.91	96.92	89.56
225	1	Plot 3	70.24	93.91	93.44	86.15

Group (g a.i./ha "RIMON" 10EC) Nominal Concentration	Time point*	Replicate	Life Stage			
			Eggs	Young larvae	Old larvae	Second spray eggs
		Plot 4	59.63	23.24	86.76	79.64
	2	Plot 3	64.51	88.75	87.51	71.98
		Plot 4	38.03	18.42	82.82	72.30
Reference, Dimilin WP, 1500 g a.i./ha	1	Plot 5	90.60	94.63	96.62	65.06
		Plot 6	89.22	51.79	96.79	87.17
	2	Plot 5	78.68	91.63	96.00	58.01
		Plot 6	81.21	44.16	91.68	77.81

* Time point 1 was 7 days after treatment and time point 2 was estimated as day 17-19 of bee development.

Reported Results: Significant effects were detected ($p<0.05$) on the development of eggs, young larvae and old larvae following the first application of "RIMON" 10EC. However, in cases where the affected brood was removed by the bees, the second generation appeared to be developing normally. This suggested that there was no long term impact on the hive viability and that the effects were transient.

There was no significant effect on the development of the eggs marked prior to the second application of "RIMON" 10EC and there was no effect on the survival of adult bees in the colony. Regular hive inspections showed that hive strength and pollen and honey reserves increased over time.

Dimilin, the reference toxicant, did not impact bees as expected. Pollen analysis showed that the level of foraging by bees in the Dimilin treated plots was considerably lower than that in the control and "RIMON"-treated plots and that, therefore, the level of exposure was likely to have been less. The

study author suggested that the reduced foraging may have been the result of repellency to Dimilin, but the fact that a significant effect of treatment with "RIMON" 10EC on brood was detected, the study design was appropriate.

Evaluation of pollen collected from hives confirmed that bees were foraging extensively on surrounding trees, with up to 60% of the pollen in the control plots coming from citrus, and up to 94% in the "RIMON" 10EC treated plots. On Dimilin treated plots, the amount of pollen coming from citrus was 40% post spray. Furthermore, analysis of pollen samples detected Novaluron in samples collected from plots treated with "RIMON" 10EC and there was no evidence of Novaluron in the control samples of pollen, indicating that there was no cross foraging by worker bees on the other treated plots in the study.

Statistical Method: Brood development data (the number of successfully developed eggs, young larvae, and old larvae out of 100 selected brood cells) were tested for normality and, subsequently, transformed using an arcsin transformation. Each treatment group was compared to the control using a one-tailed Dunnett's test (Dunnett 1955 and 1964). The statistical analyses were performed using SAS 6.11 (1989, 1996).

13. VERIFICATION OF STATISTICAL RESULTS:

The reviewer conducted Standard Two-Sample t-tests to determine if there were differences between the control and the "RIMON" 10EC treated hives for % eggs, young and old larvae, second spray eggs (for time points 1 and 2), as well as number of combs of bees, brood, honey, and pollen. With the exception of second spray eggs at time point 2, the reviewer detected no significant effects of "RIMON" 10 EC on any of these endpoints. Results of the reviewer's analysis are appended to this review.

14. REVIEWER'S COMMENTS:

The reviewer's analysis only detected a significant reduction in the second spray eggs at time point 2 for "RIMON" 10EC-exposed hives. Unlike the study author, the reviewer's analysis detected no significant effects for any other endpoint. This may be due to the different statistical methods used to detect effects. The study author's Dunnett's test detected significant reductions in % eggs, young and old larvae, and second spray eggs. However, the study author did not discriminate between analyses at the different time points; the reviewer analyzed time points separately. Despite these differences in analysis, the conclusions were identical. The results suggest that there is no significant long-lasting effect

of "RIMON" 10EC on bee development when sprayed on hives in a field setting. This study is scientifically sound and it provides useful information for risk assessment purposes. Because there are no specific guidelines for conducting this type of study, it is classified as Supplemental.

Several protocol deviations are listed on p. 17 of the study. Of these, it was mentioned that the test chemicals were stored in an air conditioned hotel room because there were no secure temperature controlled facilities for storing pesticides in the field. Pollen samples were stored in a hotel refrigerator because deep freezer space was reserved for pupae. Another deviation worth mentioning was that the first application to plot 4 was 200 g a.i. "RIMON" 10EC/ha, not 225 g a.i./ha. The study author reported that none of the deviations was considered to have an adverse effect on the outcome of the study.

Four hives were queenless on day 7 of the study, so the additional 100 egg cells were not marked in these hives. One hive was in a control plot and three hives were in the "RIMON" 10 EC treatment plots. The study author stated the loss of the queens was due to excessive handling of the hives and was not treatment-related.

The pupae extracted on day 17, the honey at the end of the study, and the pollen collected during the study were sampled for residue analysis. The pollen analysis indicate the bees had foraged in the treated area. No residues were found in the honey.

There were significant effects ($p<0.05$) on brood development (eggs, young larvae, and old larvae) after the first application of "RIMON" 10EC compared to the control. The second generation eggs appeared to develop normally. No significant differences in survival or foraging activity of "RIMON" 10EC treatment group were observed when compared to the control. There were no effects on the long-term viability of the hive. The LC_{50} could not be determined, but it was presumed to be >225 g a.i./ha.

15. REFERENCES:

OEPP/EPPO (1992) Guideline on test methods for evaluating the side-effects of plant protection products, No. 170. Honeybees. *Bulletin* OEPP/EPPO *Bulletin* 22, 203-216.

OEPP/EPPO (1993) Decision-making scheme for the environmental risk assessment of plant protection products. *Bulletin* OEPP/EPPO *Bulletin* 23, 151-165.

Oomen, P.A., De Ruijter, A. & Van Der Steen, J. (1992) Method for honeybee brood feeding tests with insect growth-regulating insecticides. *Bulletin* OEPP/EPPO *Bulletin* 22, 613-616.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

TIME POINT 1

% Eggs

```
        Standard Two-Sample t-Test

data:  x: Control in DS1 , and y: Rimon in DS1
t = 3.9421, df = 2, p-value = 0.0587
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
  -2.286639  52.286639
sample estimates:
 mean of x mean of y
    89.935    64.935
```

Young Larvae

```
        Standard Two-Sample t-Test

data:  x: Control in DS1 , and y: Rimon in DS1
t = 1.0003, df = 2, p-value = 0.4225
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -116.8452  187.6352
sample estimates:
 mean of x mean of y
     93.97    58.575
```

Old Larvae

```
        Standard Two-Sample t-Test

data:  x: Control in DS2 , and y: Rimon in DS2
t = 1.9759, df = 2, p-value = 0.1868
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
  -8.160293  22.020293
sample estimates:
 mean of x mean of y
     97.03      90.1
```

Second Spray Eggs

```
        Standard Two-Sample t-Test

data:  x: Control in DS3 , and y: Rimon in DS3
t = 2.1424, df = 2, p-value = 0.1654
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
  -9.467821  28.247821
sample estimates:
 mean of x mean of y
    92.285    82.895
```

TIME POINT 2

% Eggs

```
     Standard Two-Sample t-Test

data:  x: Control in DS4 , and y: Rimon in DS4
t = 1.7902, df = 2, p-value = 0.2153
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -38.92413  94.39413
sample estimates:
 mean of x mean of y
    79.005     51.27
```

Young Larvae

```
     Standard Two-Sample t-Test

data:  x: Control in DS5 , and y: Rimon in DS5
t = 1.0317, df = 2, p-value = 0.4106
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -115.211  187.891
sample estimates:
 mean of x mean of y
    89.925     53.585
```

Old Larvae

```
     Standard Two-Sample t-Test

data:  x: Control in DS6 , and y: Rimon in DS6
t = 2.7001, df = 2, p-value = 0.1142
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
  -5.493139  24.003139
sample estimates:
 mean of x mean of y
     94.42     85.165
```

Second Spray Eggs

```
     Standard Two-Sample t-Test

data:  x: Control in DS7 , and y: Rimon in DS7
t = 5.0513, df = 2, p-value = 0.037
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
  2.155732 26.934268
sample estimates:
 mean of x mean of y
    86.685     72.14
```

No. combs of brood

```
     Standard Two-Sample t-Test

data:  x: control in DS1 , and y: rimon in DS1
t = 1.0096, df = 2, p-value = 0.419
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -1.712483  2.762483
sample estimates:
 mean of x mean of y
     3.425       2.9
```

No. combs of honey

```
     Standard Two-Sample t-Test

data:  x: control in DS1 , and y: rimon in DS1
t = 1.0156, df = 2, p-value = 0.4167
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -2.087523  3.377523
sample estimates:
 mean of x mean of y
      5.26     4.615
```